

-39-

What is claimed is:

1. A liposome containing at least one nucleic acid encapsulated therein prepared according to a method comprising the following steps:

- (A) mixing a gel or a liquid containing gel particles with aqueous
5 medium Z1 to directly form the liposomes containing the at least one nucleic acid encapsulated therein, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid;
- (B) (i) mixing a gel or a liquid containing gel particles with aqueous
10 medium Z1 to form a curd or curdy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid;
and
(ii) mixing the curd or curdy substance with aqueous medium Z2 to
15 directly form the liposomes containing the at least one nucleic acid encapsulated therein,
- (C) (i) cooling a gel or a liquid containing gel particles to form a waxy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible
20 organic solvent and the at least one nucleic acid; and
(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes containing the at least one nucleic acid encapsulated therein;
- (D) mixing a gel or a liquid containing gel particles with aqueous
25 medium Z1 and the at least one nucleic acid to directly form the liposomes containing the at least one nucleic acid encapsulated therein, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible organic solvent;

-40-

(E) (i) mixing a gel or a liquid containing gel particles with aqueous medium Z1 and the at least one nucleic acid to form a curd or curdy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible organic solvent; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes containing the at least one nucleic acid encapsulated therein,

(F) (i) mixing a gel or a liquid containing gel particles with aqueous medium Z1 to form a curd or curdy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible organic solvent; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 and the at least one nucleic acid to directly form the liposomes containing the at least one nucleic acid encapsulated therein;

(G) (i) cooling a gel or a liquid containing gel particles to form a waxy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid; and

(ii) mixing the waxy substance with aqueous medium Z1 to directly form the liposomes containing the at least one nucleic acid encapsulated therein; or

(H) (i) cooling a gel or a liquid containing gel particles to form a waxy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible organic solvent; and

(ii) mixing the waxy substance with aqueous medium Z1 and the at least one nucleic acid to directly form the liposomes containing the at least one nucleic acid encapsulated therein;

-41-

wherein the at least one liposome-forming lipid and the at least one fusogenic lipid are the same or different; and wherein the aqueous media Z1 and Z2 are the same or different and the amount of the at least one fusogenic lipid in the gel or the liquid containing gel particles is at least about 30% by weight of the lipid content of the gel or the liquid containing gel particles.

2. The liposome of claim 1, wherein the amount of the at least one fusogenic lipid is at least about 40% by weight of the lipid content of the gel or the liquid containing gel particles.

3. The liposome of claim 2, wherein the amount of the at least one fusogenic lipid is at least about 50% by weight of the lipid content of the gel or the liquid containing gel particles.

4. The liposome of claim 3, wherein the amount of the at least one fusogenic lipid is at least about 60% by weight of the lipid content of the gel or the liquid containing gel particles.

5. The liposome of claim 4, wherein the amount of the at least one fusogenic lipid is at least about 70% by weight of the lipid content of the gel or the liquid containing gel particles.

6. The liposome of claim 5, wherein the amount of the at least one fusogenic lipid is at least about 75% by weight of the lipid content of the gel or the liquid containing gel particles.

7. The liposome of claim 6, wherein the amount of the at least one fusogenic lipid is at least about 80% by weight of the lipid content of the gel or the liquid containing gel particles.

-42-

8. The liposome of claim 1, wherein the water-miscible organic solvent in step (A) or (B) is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-butylamine, tert-butylamine, 4-butyrolactone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)-lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethylhydrazine, N-(2-hydroxyethyl)-morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2-propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2-pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

-43-

9. The liposome of claim 8, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

10. The liposome of claim 9, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

5 11. The liposome of claim 10, wherein the organic solvent is ethanol.

12. The liposome of claim 8, wherein the organic solvent is acetonitrile or acetone.

10 13. The liposome of claim 1, wherein aqueous medium Z1 and/or aqueous medium Z2 is an aqueous buffer.

14. The liposome of claim 1, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed by adding aqueous medium Z1 to the gel or the liquid.

15 15. The liposome of claim 1, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed by adding the gel or the liquid into aqueous medium Z1.

16. The liposome of claim 1, wherein the at least one nucleic acid is a DNA.

17. The liposome of claim 16, wherein the DNA is a plasmid DNA.

20 18. The liposome of claim 17, wherein the plasmid DNA is of up to about 20 kb in size.

-44-

19. The liposome of claim 18, wherein the DNA is a plasmid DNA of up to about 15 kb in size.

20. The liposome of claim 19, wherein the DNA is a plasmid DNA of up to about 10 kb in size.

5 21. The liposome of claim 18, wherein the DNA is a plasmid DNA of from about 0.5 kb to about 20 kb in size.

22. The liposome of claim 21, wherein the DNA is a plasmid DNA of from about 1 kb to about 15 kb in size.

10 23. The liposome of claim 22, wherein the DNA is a plasmid DNA of from about 2 kb to about 10 kb in size.

24. The liposome of claim 23, wherein the DNA is a plasmid DNA of from about 3 kb to about 7 kb in size.

25. The liposome of claim 1, wherein the at least one nucleic acid is an RNA.

15 26. The liposome of claim 25, wherein the RNA is an anti-sense RNA or RNA interference.

27. The liposome of claim 1, wherein the at least one nucleic acid is an oligonucleotide.

20 28. The liposome of claim 27, wherein the oligonucleotide is of about 5 to about 500 bases in size.

-45-

29. The liposome of claim 1, wherein the at least one liposome-forming lipid is selected from the group consisting of glycolipids, sphingolipids and phospholipids.

30. The liposome of claim 29, wherein the at least one liposome-forming lipid is selected from the group consisting of phospholipids.

31. The liposome of claim 30, wherein the at least one liposome-forming lipid is selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and N-acylphosphatidylethanolamine.

32. The liposome of claim 31, wherein the at least one liposome-forming lipid is selected from the group consisting of dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, dimyristoyl phosphatidylcholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1-oleoyl-2-palmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

33. The liposome of claim 1, further comprising a sterol.

34. The liposome of claim 33, wherein the sterol is cholesterol.

-46-

35. The liposome of claim 1, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

36. The liposome of claim 35, wherein the N-acyl phosphatidylethanolamine is selected from the group consisting of N-decanoyl phosphatidylethanolamine, N-undecanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine, N-tridecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

37. The liposome of claim 36, wherein the N-acyl phosphatidylethanolamine is 1,2-dioleoyl-sn-glycero-N-dodecanoyl-3-phosphoethanolamine.

38. The liposome of claim 1, wherein in the gel or the liquid containing gel particles a total amount of the at least one liposome-forming lipid and the at least one fusogenic lipid ranges from about 1% by weight of the gel or the liquid containing gel particles to the sum of the hydration limits of the at least one liposome-forming lipid and the at least one fusogenic lipid in water.

39. The liposome of claim 1, wherein in the gel or the liquid containing gel particles a total amount of the at least one liposome-forming lipid and the at least one fusogenic lipid ranges from about 5% to about 80% by weight of the gel or the liquid containing gel particles.

40. The liposome of claim 39, wherein said total amount ranges from about 10% to about 80% by weight of the gel or the liquid containing gel particles.

41. The liposome of claim 40, wherein said total amount ranges from about 15% to about 80% by weight of the gel or the liquid containing gel particles.

-47-

42. The liposome of claim 41, wherein said total amount ranges from about 20% to about 80% by weight of the gel or the liquid containing gel particles.

43. The liposome of claim 42, wherein said total amount ranges from about 30% to about 80% by weight of the gel or the liquid containing gel particles.

5 44. The liposome of claim 43, wherein said total amount ranges from about 40% to about 80% by weight of the gel or the liquid containing gel particles.

45. The liposome of claim 44, wherein said total amount ranges from about 50% to about 80% by weight of the gel or the liquid containing gel particles.

10 46. The liposome of claim 1, wherein in the gel or the liquid containing gel particles a total amount of the at least one liposome-forming lipid and the at least one fusogenic lipid ranges from about 10% to about 70% by weight of the gel or the liquid containing gel particles.

47. The liposome of claim 46, wherein said total amount ranges from about 20% to about 60% by weight of the gel or the liquid containing gel particles.

15 48. The liposome of claim 47, wherein said total amount ranges from about 30% to about 50% by weight of the gel or the liquid containing gel particles.

49. The liposome of claim 48, wherein said total amount is about 45% by weight of the gel or the liquid containing gel particles.

20 50. The liposome of claim 1, wherein aqueous medium Z1 is mixed in increments with the gel or the liquid containing gel particles, wherein the

-48-

increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

51. The liposome of claim 50, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

52. The liposome of claim 51, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

53. The liposome of claim 52, wherein the increments are up to about 40% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

54. The liposome of claim 53, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

55. The liposome of claim 54, wherein the increments are up to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

56. The liposome of claim 55, wherein the increments are up to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

-49-

57. The liposome of claim 56, wherein the increments are up to about 1 % of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

58. The liposome of claim 57, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

59. The liposome of claim 57, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

60. The liposome of claim 55, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

61. The liposome of claim 60, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

62. The liposome of claim 61, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

63. The liposome of claim 1, wherein the gel or the liquid containing gel particles comprises up to about 40 mg of the at least one nucleic acid per ml.

64. The liposome of claim 63, wherein the gel or the liquid containing gel particles comprises up to about 30 mg of the at least one nucleic acid per ml.

-50-

65. The liposome of claim 64, wherein the gel or the liquid containing gel particles comprises up to about 20 mg of the at least one nucleic acid per ml.

66. The liposome of claim 65, wherein the gel or the liquid containing gel particles comprises up to about 10 mg of the at least one nucleic acid per ml.

5 67. The liposome of claim 66, wherein the gel or the liquid containing gel particles comprises up to about 5 mg of the at least one nucleic acid per ml.

68. The liposome of claim 1, wherein the product of step (A), (B), (C), (D), (E), (F), (G) or (H) is washed with an aqueous medium by centrifugation, gel filtration or dialysis.

10 69. The liposome of claim 1, wherein the gel or the liquid containing gel particles is prepared by a method comprising the following steps:

(I) (a) (aa) mixing at least one liposome-forming lipid, the at least one fusogenic lipid, the at least one nucleic acid and a water-miscible organic solvent to form a mixture; or

15 (bb) (i) dissolving at least one liposome-forming lipid and the at least one fusogenic lipid in the water-miscible organic solvent to form an organic solution;

(ii) dissolving the at least one nucleic acid in aqueous medium X to form an aqueous solution; and

20 (iii) mixing the organic solution and aqueous solution to form a mixture; or

(b) mixing at least one liposome-forming lipid, the at least one fusogenic lipid and the water-miscible organic solvent to form a mixture; and thereafter

-51-

(II) (a) mixing the mixture of step (I)(a) with aqueous medium Y to form the gel or liquid containing gel particles; or

(b) mixing the mixture of step (I)(b) with the at least one nucleic acid and aqueous medium Y to form the gel or liquid containing gel particles,

5 wherein aqueous media X and Y are the same or different.

70. The liposome of claim 1, wherein the gel or the liquid containing gel particles is prepared by a method comprising the following steps:

(I) (a) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid, wherein the liposomes
10 are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (I)(a)(i) with the at least one nucleic acid;

(b) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in aqueous medium U,
15 wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (I)(b)(i) with the at least one nucleic acid;

(c) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid, wherein the liposomes
20 are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (I)(c)(i) with aqueous medium U and the at least one nucleic acid;

(d) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in aqueous medium U,
25 wherein the liposomes are prepared by a method other than the instant method; and

-52-

(ii) mixing the liposomes of step (I)(d)(i) with aqueous medium U and the at least one nucleic acid; or

(e) forming liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in the presence of the at least one nucleic acid by a method other than the instant method;

(II) (a) mixing the product of step (I)(b), (I)(c) or (I)(d) with the water-miscible organic solvent to form the gel or the liquid containing gel particles; or

(b) mixing the product of step (I)(a) or (I)(e) with aqueous medium V and the water-miscible organic solvent to form the gel or the liquid containing gel particles,

wherein aqueous media U and V are the same or different.

71. The liposome of claim 1, wherein the gel or liquid containing gel particles does not contain any nucleic acid condensing agent and no nucleic acid condensing agent is used in step (A), (B), (C), (D), (E), (F), (G) or (H).

72. The liposome of claim 1, wherein the gel or liquid containing gel particles does not contain any hydrating agent and no hydrating agent is used in step (A), (B), (C), (D), (E), (F), (G) or (H).

73. The liposome of claim 1, wherein a phospholipid content of the gel or the liquid containing gel particles is not 15 to 30% by weight of the gel or the liquid containing gel particles and the content of the water-miscible organic solvent is not 14 to 20% by weight of the gel or the liquid containing gel particles.

74. A method for preparing liposomes containing a nucleic acid encapsulated therein comprising the following steps:

-53-

(A) mixing a gel or a liquid containing gel particles with aqueous medium Z1 to directly form liposomes, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid, wherein
5 the at least one liposome-forming lipid and the at least one fusogenic lipid are the same or different;

(B) (i) mixing a gel or a liquid containing gel particles with aqueous medium Z1 to form a curd or curdy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one
10 fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid, wherein the at least one liposome-forming lipid and the at least one fusogenic lipid are the same or different; and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to directly form the liposomes;

15 (C) (i) cooling a gel or a liquid containing gel particles to form a waxy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible organic solvent and the at least one nucleic acid; and

(ii) mixing the waxy substance with aqueous medium Z1 to
20 directly form the liposomes containing the at least one nucleic acid encapsulated therein;

(D) mixing a gel or a liquid containing gel particles with aqueous medium Z1 and the at least one nucleic acid to directly form the liposomes containing the at least one nucleic acid encapsulated therein, wherein said gel or
25 liquid containing gel particles comprises at least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible organic solvent;

(E) (i) mixing a gel or a liquid containing gel particles with aqueous medium Z1 and the at least one nucleic acid to form a curd or curdy substance, wherein said gel or liquid containing gel particles comprises at least one liposome-

-54-

forming lipid, at least one fusogenic lipid and a water-miscible organic solvent;
and

(ii) mixing the curd or curdy substance with aqueous medium Z2 to
directly form the liposomes containing the at least one nucleic acid encapsulated
5 therein,

(F) (i) mixing a gel or a liquid containing gel particles with aqueous
medium Z1 to form a curd or curdy substance, wherein said gel or liquid
containing gel particles comprises at least one liposome-forming lipid, at least one
fusogenic lipid and a water-miscible organic solvent; and

10 (ii) mixing the curd or curdy substance with aqueous medium Z2
and the at least one nucleic acid to directly form the liposomes containing the at
least one nucleic acid encapsulated therein;

(G) (i) cooling a gel or a liquid containing gel particles to form a
waxy substance, wherein said gel or liquid containing gel particles comprises at
15 least one liposome-forming lipid, at least one fusogenic lipid, a water-miscible
organic solvent and the at least one nucleic acid; and

(ii) mixing the waxy substance with aqueous medium Z1 to
directly form the liposomes containing the at least one nucleic acid encapsulated
therein; or

20 (H) (i) cooling a gel or a liquid containing gel particles to form a
waxy substance, wherein said gel or liquid containing gel particles comprises at
least one liposome-forming lipid, at least one fusogenic lipid and a water-miscible
organic solvent; and

(ii) mixing the waxy substance with aqueous medium Z1 and the
25 at least one nucleic acid to directly form the liposomes containing the at least one
nucleic acid encapsulated therein;

wherein the aqueous media Z1 and Z2 are the same or different and an
amount of the at least one fusogenic lipid is at least 30% by weight of a lipid
content of the gel or the liquid containing gel particles.

-55-

75. The method of claim 74, wherein the amount of the at least one fusogenic lipid is at least 40% by weight of a lipid content of the gel or the liquid containing gel particles.

5 76. The method of claim 75, wherein the amount of the at least one fusogenic lipid is at least 50% by weight of a lipid content of the gel or the liquid containing gel particles.

77. The method of claim 76, wherein the amount of the at least one fusogenic lipid is at least 60% by weight of a lipid content of the gel or the liquid containing gel particles.

10 78. The method of claim 77, wherein the amount of the at least one fusogenic lipid is at least 70% by weight of a lipid content of the gel or the liquid containing gel particles.

79. The method of claim 78, wherein the amount of the at least one fusogenic lipid is at least 75% by weight of a lipid content of the gel or the liquid containing gel particles.

15

80. The method of claim 79, wherein the amount of the at least one fusogenic lipid is at least 80% by weight of a lipid content of the gel or the liquid containing gel particles.

81. The method of claim 74, wherein the water-miscible organic solvent is selected from the group consisting of acetaldehyde, acetone, acetonitrile, allyl alcohol, allylamine, 2-amino-1-butanol, 1-aminoethanol, 2-aminoethanol, 2-amino-2-ethyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, 3-aminopentane, N-(3-aminopropyl)morpholine, benzylamine, bis(2-ethoxyethyl) ether, bis(2-

20

-56-

hydroxyethyl) ether, bis(2-hydropropyl) ether, bis(2-methoxyethyl) ether, 2-bromoethanol, meso-2,3-butanediol, 2-(2-butoxyethoxy)-ethanol, butylamine, sec-butylamine, tert-butylamine, 4-butyrolactone, 2-chloroethanol, 1-chloro-2-propanol, 2-cyanoethanol, 3-cyanopyridine, cyclohexylamine, diethylamine, 5 diethylenetriamine, N,N-diethylformamide, 1,2-dihydroxy-4-methylbenzene, N,N-dimethylacetamide, N,N-dimethylformamide, 2,6-dimethylmorpholine, 1,4-dioxane, 1,3-dioxolane, dipentaerythritol, ethanol, 2,3-epoxy-1-propanol, 2-ethoxyethanol, 2-(2-ethoxyethoxy)-ethanol, 2-(2-ethoxyethoxy)-ethyl acetate, ethylamine, 2-(ethylamino)ethanol, ethylene glycol, ethylene oxide, ethylenimine, ethyl(-)- 10 lactate, N-ethylmorpholine, ethyl-2-pyridine-carboxylate, formamide, furfuryl alcohol, furfurylamine, glutaric dialdehyde, glycerol, hexamethylphosphoramide, 2,5-hexanedione, hydroxyacetone, 2-hydroxyethylhydrazine, N-(2-hydroxyethyl)-morpholine, 4-hydroxy-4-methyl-2-pentanone, 5-hydroxy-2-pentanone, 2-hydroxypropionitrile, 3-hydroxypropionitrile, 1-(2-hydroxy-1-propoxy)-2- 15 propanol, isobutylamine, isopropylamine, 2-isopropylamino-ethanol, 2-mercaptoethanol, methanol, 3-methoxy-1-butanol, 2-methoxyethanol, 2-(2-methoxyethoxy)-ethanol, 1-methoxy-2-propanol, 2-(methylamino)-ethanol, 1-methylbutylamine, methylhydrazine, methyl hydroperoxide, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, N-methylpyrrolidine, N-methyl-2- 20 pyrrolidinone, morpholine, nicotine, piperidine, 1,2-propanediol, 1,3-propanediol, 1-propanol, 2-propanol, propylamine, propyleneimine, 2-propyn-1-ol, pyridine, pyrimidine, pyrrolidine, 2-pyrrolidinone and quinoxaline.

82. The method of claim 81, wherein the organic solvent is acetonitrile, acetone or a C₁-C₃ alcohol.

25 83. The method of claim 82, wherein the organic solvent is methanol, ethanol, 1-propanol, 2-propanol, ethylene glycol or propylene glycol.

-57-

84. The method of claim 83, wherein the organic solvent is ethanol, 1-propanol or 2-propanol.

85. The method of claim 84, wherein the organic solvent is ethanol.

86. The method of claim 82, wherein the organic solvent is acetone.

5 87. The method of claim 74, wherein aqueous medium Z1 and/or aqueous medium Z2 is an aqueous buffer.

88. The method of claim 74, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed by adding aqueous medium Z1 to the gel or the liquid.

10 89. The method of claim 74, wherein the gel or the liquid containing gel particles and aqueous medium Z1 are mixed by adding the gel or the liquid into aqueous medium Z1.

90. The method of claim 74, wherein the at least one nucleic acid is a DNA.

15 91. The method of claim 90, wherein the DNA is a plasmid DNA.

92. The method of claim 91, wherein the plasmid DNA is of up to about 20 kb in size.

93. The method of claim 92, wherein the DNA is a plasmid DNA of up to about 15 kb in size.

-58-

94. The method of claim 93, wherein the DNA is a plasmid DNA of up to about 10 kb in size.

95. The method of claim 92, wherein the DNA is a plasmid DNA of from about 0.5 kb to about 20 kb in size.

5 96. The method of claim 95, wherein the DNA is a plasmid DNA of from about 1 kb to about 15 kb in size.

97. The method of claim 96, wherein the DNA is a plasmid DNA of from about 2 kb to about 10 kb in size.

10 98. The method of claim 97, wherein the DNA is a plasmid DNA of from about 3 kb to about 7 kb in size.

99. The method of claim 74, wherein the at least one nucleic acid is an RNA.

100. The method of claim 74, wherein the at least one nucleic acid is an oligonucleotide.

15 101. The method of claim 100, wherein the oligonucleotide is of about 5 to about 500 bases in size.

102. The method of claim 74, wherein the at least one liposome-forming lipid is selected from the group consisting of glycolipids, sphingolipids and phospholipids.

-59-

103. The method of claim 102, wherein the at least one liposome-forming lipid is selected from the group consisting of phospholipids.

104. The method of claim 103, wherein the at least one liposome-forming lipid is selected from the group consisting of phosphatidylcholine,
5 phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and N-acylphosphatidylethanolamine.

105. The method of claim 104, wherein the at least one liposome-forming lipid is selected from the group consisting of dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, dimyristoyl
10 phosphatidylcholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)],
15 1-oleoyl-2-palmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)], N-decanoyl phosphatidylethanolamine, N-dodecanoyl phosphatidylethanolamine and N-tetradecanoyl phosphatidylethanolamine.

106. The method of claim 74, further comprising adding a sterol in step (A), (B), (C), (D), (E), (F), (G) or (H).

20 107. The method of claim 106, wherein the sterol is cholesterol.

108. The method of claim 74, wherein the at least one fusogenic lipid is selected from the group consisting of N-acyl phosphatidylethanolamine.

-60-

109. The method of claim 108, wherein the N-acyl
phosphatidylethanolamine is selected from the group consisting of N-decanoyl
phosphatidylethanolamine, N-decanoyl phosphatidylethanolamine, N-undecanoyl
phosphatidylethanolamine, N-tridecanoyl phosphatidylethanolamine and N-
5 tetradecanoyl phosphatidylethanolamine.

110. The method of claim 109, wherein the N-acyl
phosphatidylethanolamine. is 1,2-dioleoyl-sn-glycero-N-dodecanoyl-3-
phosphoethanolamine.

111. The method of claim 74, wherein in the gel or the liquid containing
10 gel particles a total amount of the at least one liposome-forming lipid and the at
least one fusogenic lipid ranges from about 1 % by weight of the gel or the liquid
containing gel particles to the sum of the hydration limits of the at least one
liposome-forming lipid and the at least one fusogenic lipid in water.

112. The method of claim 74, wherein in the gel or the liquid containing
15 gel particles a total amount of the at least one liposome-forming lipid and the at
least one fusogenic lipid ranges from about 5 % to about 80 % by weight of the the
gel or the liquid containing gel particles.

113. The method of claim 112, wherein said total amount ranges from
about 10% to about 80% by weight of the the gel or the liquid containing gel
20 particles.

114. The method of claim 113, wherein said total amount ranges from
about 15% to about 80% by weight of the the gel or the liquid containing gel
particles.

-61-

115. The method of claim 114, wherein said total amount ranges from about 20% to about 80% by weight of the the gel or the liquid containing gel particles.

5 116. The method of claim 115, wherein said total amount ranges from about 30% to about 80% by weight of the the gel or the liquid containing gel particles.

117. The method of claim 116, wherein said total amount ranges from about 40% to about 80% by weight of the the gel or the liquid containing gel particles.

10 118. The method of claim 117, wherein said total amount ranges from about 50% to about 80% by weight of the the gel or the liquid containing gel particles.

15 119. The method of claim 118, wherein said total amount ranges from about 10% to about 70% by weight of the the gel or the liquid containing gel particles.

120. The method of claim 119, wherein said total amount ranges from about 20% to about 60% by weight of the the gel or the liquid containing gel particles.

20 121. The method of claim 120, wherein said total amount ranges from about 30% to about 50% by weight of the the gel or the liquid containing gel particles.

-62-

122. The method of claim 121, wherein said total amount is about 45% by weight of the the gel or the liquid containing gel particles.

123. The method of claim 74, wherein aqueous medium Z1 is mixed in increments with the gel or the liquid containing gel particles, wherein the
5 increments are up to about 100% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

124. The method of claim 123, wherein the increments are up to about 80% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

10 125. The method of claim 124, wherein the increments are up to about 60% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

126. The method of claim 125, wherein the increments are up to about 40% of the weight of the gel or the liquid containing gel particles before the gel or
15 the liquid is mixed with any aqueous medium Z1.

127. The method of claim 126, wherein the increments are up to about 20% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

128. The method of claim 127, wherein the increments are up to about
20 10% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

-63-

129. The method of claim 128, wherein the increments are up to about 5 % of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

5 130. The method of claim 129, wherein the increments are up to about 1 % of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

131. The method of claim 130, wherein the increments are up to about 0.5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

10 132. The method of claim 131, wherein the increments are up to about 0.1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

133. The method of claim 128, wherein the increments are from about 0.001% to about 10% of the weight of the gel or the liquid containing gel particles
15 before the gel or the liquid is mixed with any aqueous medium Z1.

134. The method of claim 133, wherein the increments are from about 0.001% to about 5% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

20 135. The method of claim 134, wherein the increments are from about 0.001% to about 1% of the weight of the gel or the liquid containing gel particles before the gel or the liquid is mixed with any aqueous medium Z1.

-64-

136. The method of claim 74, wherein the gel or the liquid containing gel particles comprises up to about 40 mg of the at least one nucleic acid per ml.

137. The method of claim 136, wherein the gel or the liquid containing gel particles comprises up to about 30 mg of the at least one nucleic acid per ml.

5 138. The method of claim 137, wherein the gel or the liquid containing gel particles comprises up to about 20 mg of the at least one nucleic acid per ml.

139. The method of claim 138, wherein the gel or the liquid containing gel particles comprises up to about 10 mg of the at least one nucleic acid per ml.

10 140. The method of claim 139, wherein the gel or the liquid containing gel particles comprises up to about 5 mg of the at least one nucleic acid per ml.

141. The method of claim 74, wherein the product of step (A), (B), (C), (D), (E), (F), (G) or (H) is washed with an aqueous medium by centrifugation, gel filtration or dialysis.

15 142. The method of claim 74, wherein the gel or the liquid containing gel particles is prepared by a method comprising the following steps:

(I) (a) (aa) mixing at least one liposome-forming lipid, the at least one fusogenic lipid, the at least one nucleic acid and a water-miscible organic solvent to form a mixture; or

20 (bb) (i) dissolving at least one liposome-forming lipid and the at least one fusogenic lipid in the water-miscible organic solvent to form an organic solution;

 (ii) dissolving the at least one nucleic acid in aqueous medium X to form an aqueous solution; and

-65-

(iii) mixing the organic solution and aqueous solution to form a mixture; or

(b) mixing at least one liposome-forming lipid, the at least one fusogenic lipid and the water-miscible organic solvent to form a mixture; and
5 thereafter

(II) (a) mixing the mixture of step (I)(a) with aqueous medium Y and optionally the at least one nucleic acid to form the gel or liquid containing gel particles; or

(b) mixing the mixture of step (I)(b) with the at least one nucleic acid and aqueous medium Y to form the gel or liquid containing gel particles,
10 wherein aqueous media X and Y are the same or different.

143. The method of claim 74, wherein the gel or the liquid containing gel particles is prepared by a method comprising the following steps:

(I) (a) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid, wherein the liposomes
15 are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (I)(a)(i) with the at least one nucleic acid;

(b) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in aqueous medium U,
20 wherein the liposomes are prepared by a method other than the instant method; and

(ii) mixing the liposomes of step (I)(b)(i) with the at least one nucleic acid;

(c) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid, wherein the liposomes
25 are prepared by a method other than the instant method; and

-66-

(ii) mixing the liposomes of step (I)(c)(i) with aqueous medium U and the at least one nucleic acid;

(d) (i) providing liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in aqueous medium U, wherein the liposomes are prepared by a method other than the instant method;
5 and

(ii) mixing the liposomes of step (I)(d)(i) with aqueous medium U and the at least one nucleic acid;

(e) forming liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in the presence of the at least one nucleic acid by a method other than the instant method; or
10

(f) forming liposomes comprising the at least one liposome-forming lipid and the at least one fusogenic lipid in aqueous medium U in the presence of the at least one nucleic acid by a method other than the instant method; and
15 thereafter

(II) (a) mixing the product of step (I)(b), (I)(c), (I)(d) or (I)(f) with the water-miscible organic solvent to form the gel or the liquid containing gel particles; or

(b) mixing the product of step (I)(a) or (I)(e) with aqueous medium U and the water-miscible organic solvent to form the gel or the liquid containing gel particles,
20

wherein aqueous media U and V are the same or different.

144. The method of claim 74, wherein the gel or liquid containing gel particles does not contain any nucleic acid condensing agent and no nucleic acid condensing agent is used in step (A), (B), (C), (D), (E), (F), (G) or (H).
25

-67-

145. The method of claim 74, wherein no hydrating agent is used in in step (A), (B), (C), (D), (E), (F), (G) or (H) and wherein the gel or the liquid containing gel particles does not contain a hydrating agent.

5 146. The method of claim 74, wherein a phospholipid content of the gel or the liquid containing gel particles is not 15 to 30% by weight of the gel or the liquid and the content of the water-miscible organic solvent is not 14 to 20% by weight of the gel or the liquid containing gel particles.

147. A method for transfecting a eukaryotic cell with a plasmid DNA, comprising the following steps:

10 (a) providing the liposome of claim 1, wherein the at least one nucleic acid is the plasmid DNA; and thereafter

(b) contacting the cell with the liposome to transfect the cell with the plasmid DNA.

148. The method of claim 147, wherein step (b) is conducted by

15 incubating the cell with the liposome at 37°C.

149. A method for transfecting a eukaryotic cell with a plasmid DNA in a eukaryotic subject in need of the transfection, said method comprising the following steps:

(a) providing the liposome of claim 1, wherein the at least one nucleic

20 acid is the plasmid DNA; and thereafter

(b) administering the liposome in the eukaryotic subject.

150. The method of claim 149, wherein the liposome is administered intravenously in the eukaryotic subject.

-68-

151. The method of claim 150, wherein the eukaryotic subject is a human.

152. The method of claim 151, wherein the eukaryotic subject is a human in need of gene therapy and the plasmid DNA contains a gene necessary for the gene therapy.

5 153. The liposome of claim 1, wherein in the gel or the liquid containing gel particles a total amount of the at least one liposome-forming lipid and the at least one fusogenic lipid ranges from about 5% to about 95% by weight of the gel or the liquid containing gel particles.

10 154. The liposome of claim 153, wherein said total amount ranges from about 10% to about 95% by weight of the gel or the liquid containing gel particles.

155. The liposome of claim 154, wherein said total amount ranges from about 15% to about 95% by weight of the gel or the liquid containing gel particles.

156. The liposome of claim 155, wherein said total amount ranges from about 20% to about 95% by weight of the gel or the liquid containing gel particles.

15 157. The liposome of claim 156, wherein said total amount ranges from about 30% to about 95% by weight of the gel or the liquid containing gel particles.

158. The liposome of claim 157, wherein said total amount ranges from about 40% to about 95% by weight of the gel or the liquid containing gel particles.

20 159. The liposome of claim 158, wherein said total amount ranges from about 50% to about 95% by weight of the gel or the liquid containing gel particles.

-69-

160. The liposome of claim 1, wherein in the gel or the liquid containing gel particles a total amount of the at least one liposome-forming lipid and the at least one fusogenic lipid ranges from about 5% to about 90% by weight of the gel or the liquid containing gel particles.

5 161. The liposome of claim 160, wherein said total amount ranges from about 10% to about 90% by weight of the gel or the liquid containing gel particles.

162. The liposome of claim 160, wherein said total amount ranges from about 15% to about 90% by weight of the gel or the liquid containing gel particles.

10 163. The liposome of claim 162, wherein said total amount ranges from about 20% to about 90% by weight of the gel or the liquid containing gel particles.

164. The liposome of claim 163, wherein said total amount ranges from about 30% to about 90% by weight of the gel or the liquid containing gel particles.

165. The liposome of claim 164, wherein said total amount ranges from about 40% to about 90% by weight of the gel or the liquid containing gel particles.

15 166. The liposome of claim 165, wherein said total amount ranges from about 50% to about 90% by weight of the gel or the liquid containing gel particles.

167. The liposome of claim 166, wherein said total amount ranges from about 60% to about 90% by weight of the gel or the liquid containing gel particles.

20 168. The liposome of claim 73, wherein the content of the water-miscible organic solvent is not 14 to 20% by weight of the gel or the liquid.